IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Marlene M. DARFLER., et al.)	Confirmation No: 9373
)	
Application Serial No.: 10/796,288)	Group Art Unit: 1657
)	
Filed: March 10, 2004)	Examiner: Kailash Srivastava, Ph.D.

For: LIQUID TISSUE PREPARATION FROM HISTOPATHOLOGICALLY PROCESSED BIOLOGICALLY SAMPLES, TISSUES AND CELLS

United States Patent and Trademark Office
Randolph Building
401 Dulany Street
Alexandria, Virginia 22314

Declaration under 37 C.F.R. § 1.132

I, Dr. Anirban Maitra, declare and say:

1. I am an Associate Professor of Pathology and Oncology at the Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins University School of Medicine. I am also Editor-in-Chief of the scientific journal *Current Molecular Medicine.*

2. A copy of my *Curriculum Vitae* is appended below as APPENDIX A.

3. I make the following statement regarding the use of organic solvents for the removal of paraffin from standard tissue sections and the use of the term "organic solvents" in reference to an entire collection of reagents well known to those skilled in the art and science of pathology.

4. Paraffin has been used for many decades as an embedding medium in the preparation of tissue specimens for sectioning in a microtome to produce specimen sections for histological studies. Such embedding processes are well known in the field and generally include the well known steps of: specimen fixation; dehydration; clearing; paraffin infiltration or impregnation; embedding in a block of paraffin; slicing the block and specimen into sections; mounting the sections on slides; removing the paraffin with organic solvents employed for this purpose (deparaffinizing); and staining the sections prior to microscopic analysis.

5. Fixation is performed to preserve the structure of the tissue. This process provides rigidity to the tissue, making it easier to section. Common fixatives used include formalin and glutaraldehyde. Once placed in the fixative, covalent bonds are formed between the fixative and the amine groups of the tissue proteins, cross-linking the proteins. Once fixation has been completed, the sample is embedded prior to sectioning. The primary purpose of the embedding

medium is to permit the specimens to be sectioned and mounted on glass slides in their natural state, and removal of paraffin by organic solvents is necessary for further histological analysis.

6. Xylene is the most commonly used organic solvent to solubilize paraffin for deparaffinization of specimen sections, however, other organic solvents that have been used in the past or are currently used for removal of paraffin from thin tissue sections include chloroform, benzene, toluene, hexane, and heptanes. The physical and chemical properties of organic solvents used to solubilize paraffin for deparaffinization of samples would have been well known one having ordinary skill in the art at the time the captioned application was filed. For example, the solvent should be sufficiently non-polar to dissolve paraffin without causing chemical damage to the tissue proteins.

7. In a typical process, a microscope slide-mounted specimen is immersed in a xylene bath until the paraffin is solubilized. The deparaffinized specimen is then washed with a series of alcohol solutions of decreasing alcohol concentration, typically as baths in which the specimen is immersed, to remove xylene, before a final wash with water. The nature and identity of a wide variety of organic solvents that are used for paraffin removal from tissue sections are well known to those skilled in the art, and the use of the term "organic solvents" to describe an entire collection of reagents for removal of paraffin from tissue sections is well understood by those skilled in the art.

8. Drip column fractionation is a term that is in common usage in biochemical and chemical research, and its meaning is clearly understood in the art. Specifically, the term refers to a method of separating molecules, such as proteins, by passing a liquid sample containing the molecules to be separated down a column containing a separation medium, such as an ion-exchange resin, and collecting the eluate that drips from the column. The different molecules present in the sample bind to the separation medium to a differing degree and therefore elute from the column in different fractions. I have appended four excerpts from the scientific literature that refer to drip column techniques (see APPENDIX B) used by chemists and biochemists to separate samples. There excerpts clearly demonstrate that the term "drip column fractionation" has a clear meaning in the art.

9. Reference #1 describes drip columns in the last paragraph of Section 2:

> "If a drip column format is used, the displacer is allowed to pass into the column bed and the flow is then halted (e.g. by capping the column outlet). After a period of equilibration (15-30 minutes) the dissociated proteins are flushed out by application of more elution buffer. This step can be repeated until protein is absent from the eluted fractions."

10. Reference #2, US Patent 5,336,412 in the Background of the Invention refers to

> "Conventional gel-chromatography "drip" columns..."

11. Reference #3 is a commercial catalog that states:

> "The easy-to-use Zeba Spin Format dramatically improves results over standard drip-column methodologies, eliminating the need to wait for samples to emerge by gravity flow and the need to monitor fractions for protein recovery."

12. Reference #4 is an excerpt from the book ":CDNA Library Protocols" by Ian G. Cowell and Caroline A. Austin (Humana Press, 1996, ISBN:089603383X) that describes drip column preparation.

13. All statements made herein of my knowledge are true and all statements made on information and belief are believed to be true; and further, these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any document or any registration resulting therefrom.

Date: 08/14/08

Dr. Anirban Maitra

APPENDIX A

Curriculum Vitae – Anirban Maitra

CURRICULUM VITAE

Anirban Maitra

ANIRBAN MAITRA, M.B.B.S.

DEMOGRAPHIC INFORMATION

Current Appointments:

University:	Associate Professor of Pathology Associate Professor of Oncology (secondary) Affiliate, McKusick-Nathans Institute of Genetic Medicine
Hospital:	Pathologist, The Johns Hopkins Hospital

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Education and Training:

1990-1996	Bachelor of Medicine, Bachelor of Surgery (MBBS), All Indian Institute of Medical Sciences, New Delhi, India
1996-1998	Residency in Anatomic Pathology, University of Texas Southwestern Medical Center, Dallas
1998-1999	Research Fellow, Molecular Pathology, University of Texas Southwestern Medical Center, Dallas
1999-2000	Clinical Fellow, Pediatric Pathology, University of Texas Southwestern Medical Center, Dallas
1999-2001	Residency in Anatomic Pathology, University of Texas Southwestern Medical Center, Dallas
2000-2001	Clinical/Research Fellow, Gastrointestinal Pathology, Johns Hopkins University School of Medicine, Baltimore.

Professional Experience:

2002-2003	Instructor, Gastrointestinal Pathology, Johns Hopkins University School of Med, Baltimore.
2002-	Affiliate, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins Univ., Baltimore
2003-2005	Assistant Prof, Pathology, Johns Hopkins University School of Medicine, Baltimore.
2003-2005	Assistant Prof, Oncology, Johns Hopkins University School of Medicine, Baltimore.
2005-	Graduate Faculty, Pathobiology Program, Johns Hopkins University School of Med, Baltimore
2006-	Assoc Professor, Pathology, Johns Hopkins Univ School of Medicine, Baltimore.
2006	Assoc Professor, Oncology, Johns Hopkins University School of Medicine Baltimore.

RESEARCH ACTIVITIES: Peer-reviewed Scientific Publications

First and Last Author Papers

1. **Maitra, A.**, Hirany, S. V. and Jialal, I. Comparison of two assays for measuring LDL cholesterol. *Clin Chem* 43:1040-7, 1997.

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12. **Maitra**, A., Ramnani, D.M., Margraf, L., Gazdar, A.F. Synchronous Wilms tumor and fibrolamellar hepatocellular carcinoma : report of a case. *Ped Develop Pathol* 3:492-6, 2000

13. **Maitra**, A., Krueger, J.E., Tascilar, M., Klimstra, D.S., Hruban, R.H., Angeles-Angeles, A., and Albores-Saavedra, J. Carcinoid tumors of the extrahepatic bile ducts: a study of seven cases. *Am J Surg Pathol* 24:1501-10, 2000.

14. **Maitra**, A., Schneider, N.R., Weinberg, A.G, Patterson, K. Detection of t(11;22)(q24;q12) translocation and EWS-FLI-1 fusion transcript in a case of solid pseudopapillary tumor of the pancreas. *Pediatr Develop Pathol* 3:603-5; 2000

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Collaborative Papers

1. Yashima, K., **Maitra, A.**, Rogers, B. B., Timmons, C. F., Rathi, A., Pinar, H., et al. Expression of the RNA component of telomerase during human development and differentiation. *Cell Growth Diff*, 9:805-13, 1998.

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41. Hansel, D.E., Meeker, A.K., Lillemoe, K.D., Shulick, R., Hruban, R.H., **Maitra, A.,** Argani, P. Telomere Length Variation in Biliary Tract Metaplasia, Dysplasia and Carcinoma (92nd United States and Canadian Academy of Pathology Meeting, Washington, DC, 2003)

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52. Swierczynski, S.L., Argani, P., Iacobuzio-Donahue, C.A., Ashfaq, R., Cameron, J.L., Yeo, C.J., Rahman, A., Hruban, R.H., **Maitra, A.** Development and Validation of Tissue Microarrays in Pancreatic Cancer (92nd United States and Canadian Academy of Pathology Meeting, Washington, DC, 2003)

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58. Henke, R. T., **Maitra, A.**, Gvozdjan, D., Tassi, E., McDonnell, K., Wellstein, A. Expression of newly discovered metastasis genes in tissue microarrays of gastrointestinal cancers is predictive of metastasis status (94th American Association of Cancer Research Meeting, Washington, DC, 2003)

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60. House, M.G., Wistuba, I.I., Argani, P., Guo, M., Schulick, R.D., Hruban, R.H., Herman, J.G., **Maitra, A.** Hypermethylation of tumor suppressor genes in gallbladder cancer (Society of Surgical Oncology Annual Meeting, Los Angeles, 2003)

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63. Amador, M. L., **Maitra, A.**, Gruenwald, V., Peralba, J. M., Hidalgo, M. Determinants of resistance to OSI-774 in billiary tract carcinoma cell lines. (American Society of Clinical Oncology Annual Meeting, Chicago, IL 2003)

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67. **Maitra, A.**, Cao, D., Albores-Saavedra, J., Klimstra, D., Hruban, R.H. Expression of epithelial markers in nonductal neoplasms of the pancreas. (93rd United States and Canadian Academy of Pathology Meeting, Vancouver, British Columbia, 2004)

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70. Klimstra, D.S., Takaori, K., Hruban, R.H., Adsay, N.V., Albores-Saavedra, J., Biankin, A.V., Biankin, S.A., Compton, C.C., Fukushima, N., Furukawa, T., Goggins, M., Kato, Y., Kloeppel, G., Longnecker, D., Luettges, J., **Maitra, A.**, Offerhaus, G.J., Shimizu, M., Yonezawa, S. Consensus Criteria for the Classification of Pancreatic Intraepithelial Neoplasia (PanIN) and Intraductal Papillary Mucinous Neoplasms (IPMNs). (93rd United States and Canadian Academy of Pathology Meeting, Vancouver, British Columbia, 2004)

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72. Kumar, S.K., Roy, I., Denmeade, S.R., Isaacs, J.T., **Maitra, A.**, Khan, S.R. Nanoparticles as novel vectors for targeted drug delivery to prostate cancer. (95th American Association of Cancer Research Meeting, Orlando, FL, 2004)

73. Hansel, D.E., Dhara, S., **Maitra, A.**, Montgomery, E. *CDC2/p34* upregulation and expression in a progression model of esophageal adenocarcinomas. (95th American Association of Cancer Research Meeting, Orlando, FL, 2004)

74. Bashyam, M.D., Kim, Y.K., **Maitra, A.**, Pollack, J.R. Localization of novel pancreatic cancer genes by CGH on cDNA microarrays. (95th American Association of Cancer Research Meeting, Orlando, FL, 2004)

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76. Hingorani, S.R., Petricoin, E.F., **Maitra, A.**, King, C., Jacobetz, M.A., Yoshiya, K., Crawford, H.C., Putt, M.E., Jacks, T., Konieczny, S. F., Wright, C.V., Hruban, R.H., Lowy, A., Tuveson, D.A. Endogenous *KRAS*^{G12D} expression induces pancreatic intraepithelial neoplasia (PanIN) in mice with a definable proteomic signature. (95th American Association of Cancer Research Meeting, Orlando, FL, 2004)

77. Hansel, D, Dhara, S, Huang, R, Deasel, M, Ashfaq, R, Shimada, Y, Bernstein, H, Harmon, J, Brock, M, Forestiere, A, Washington, K, **Maitra, A**, Montgomery, E. CDC2/CDK1 Expression in Esophageal Adenocarcinoma and Precursor Lesions Serves as a Diagnostic and Cancer Progression Marker and Potential Novel Drug Target (94th Annual Meeting of the United States and Canadian Academy of Pathology, San Antonio, Texas, March 2005)

78. Montgomery, E., Cunningham, S., Schulick, R., Yeo, C., Haque, R., Hammoud, S., Hustinx, S., Kim, M., Iacobuzio-Donahue, C., Ashfaq, R., Kamangar, F., **Maitra, A.** Identification of Novel Cellular Targets in the Progression Model of Gastric Adenocarcinoma (94th Annual Meeting of the United States and Canadian Academy of Pathology, San Antonio, Texas, March 2005)

79. Yang, G-Y, Dilworth, H.P., Ashfaq, R., Hruban, R.H., **Maitra, A.** Tissue Transglutaminase II: A Biomarker of Pancreatic Ductal Adenocarcinoma (94th Annual Meeting of the United States and Canadian Academy of Pathology, San Antonio, Texas, March 2005)

80. Chandrasekharan, A., Shi, C., Thuluvath, P.J., Wistuba, I.I., Karikari, C.A., Argani, P., Goggins, M.G., Eshleman, J.R., **Maitra, A.** Ultrasensitive Detection of KRAS Mutations in Bile and Serum from Patients with Biliary Cancer Using LigAmp Technology (94th Annual Meeting of the United States and Canadian Academy of Pathology, San Antonio, Texas, March 2005)

81. Hustinx, S.R., Hruban, R.H., Leoni, L.M., Argani, P., Ashfaq, R., Goggins, M.G., Kern, S., **Maitra, A.** Homozygous Deletion of the MTAP Gene in Invasive Adenocarcinoma of the Pancreas and in Periampullary Cancer: A Potential New Target for Therapy (94th Annual Meeting of the United States and Canadian Academy of Pathology, San Antonio, Texas, March 2005)

82. Hustinx, S.R., **Maitra, A.**, Leoni, L.M., Ashfaq, R., Goggins, M.G., Iacobuzio-Donahue, C., Kern, S., Hruban, R.H. Concordant Loss MTAP and p16/CDKN2A Expression in Pancreatic Intraepithelial Neoplasia: Evidence of Homozygous Deletion in a Non-Invasive Precursor Lesion (94th Annual Meeting of the United States and Canadian Academy of Pathology, San Antonio, Texas, March 2005)

83. Hustinx, S.R., Cao, D., Hruban, R.H., Sato, N., Martin, S., Sudhir, D., Iacobuzio-Donahue, C., Kern, S., Goggins, M.G., Pandey, A., **Maitra, A.** Differentially Expressed Genes in Pancreatic Ductal Adenocarcinomas Identified through Serial Analysis of Gene Expression: An Update (94th Annual Meeting of the United States and Canadian Academy of Pathology, San Antonio, Texas, March 2005)

84. **Maitra, A.**, Cao, D., Lee, K., Itami, A., Hruban, R.H., Ouellette, M. The Tubulin Beta-4 Polypeptide (TUBB4), a Marker of Resistance to Taxanes, Is Overexpressed in Pancreatic Intraepithelial Neoplasia and Pancreatic Ductal Adenocarcinoma (94th Annual Meeting of the United States and Canadian Academy of Pathology, San Antonio, Texas, March 2005)

85. Zhang, H., Basturk, O., Othman, M., Cheng, J.D., Khayyata, S.H., **Maitra, A.**, Hruban, R.H., Adsay, N.V. Expression of MUC1, MUC2, MUC5AC and CDX2 in Carcinomas of the Gallbladder and Extrahepatic Bile Duct (94th Annual Meeting of the United States and Canadian Academy of Pathology, San Antonio, Texas, March 2005)

86. Cao, D., Kassaui, K., Argani, P., Neumann, C., Ho, L., Abbruzzese, J., Ouellette, M., **Maitra, A.** Expression of Two Novel Tumor Markers, Maspin and Tubulin beta Polypeptide (TUBB), in Biliary Cancers (94th Annual Meeting of the United States and Canadian Academy of Pathology, San Antonio, Texas, March 2005)

87. Henke, R.T., Kim, S.E., Fang, W.J., **Maitra, A.**, Wellstein, A. Outcome in Patients with Colorectal Adenocarcinoma in Relation to the Expression of the Growth Factor Pleiotrophin and Its Receptor Anaplastic Lymphoma Kinase (Poster presentation at the 94th Annual Meeting of the United States and Canadian Academy of Pathology, San Antonio, Texas, March 2005)

88. Powell, E.L., Montgomery, E., Leoni,L., **Maitra, A.** Concordant Loss of MTAP and p16/CDKN2A Expression in Barrett Esophagus and Adenocarcinoma: Evidence of Homozygous Deletion in Non-Invasive Precursor Lesions (94th Annual Meeting of the United States and Canadian Academy of Pathology, San Antonio, Texas, March 2005)

89. **Maitra, A.**, Cao, D., Neumann, C., Abbruzzese, J., Ho, L. Aberrant Expression of Maspin in Idiopathic Inflammatory Bowel Disease Is Associated with Disease Activity and Neoplastic Transformation (94th Annual Meeting of the United States and Canadian Academy of Pathology, San Antonio, Texas, March 2005)

90. Salaria, S. Cronin, M., Kim, E., Zhang, Q., **Maitra, A.**, Neumann, C., Hruban, R.H., Goggins, M.G., Abbruzzese, J., Ho, L. Maspin Is Overexpressed in the Majority of Pancreatic Ductal Adenocarcinomas and Pancreatic Intraepithelial Neoplasia (PanIN) Lesions: A Potential Biomarker for Early Detection (94th Annual Meeting of the United States and Canadian Academy of Pathology, San Antonio, Texas, March 2005)

91. Hansel, D.E., **Maitra, A.**, House, M.G., Yeo, C.J., Ali, S.Z. Differential Expression of Neuroendocrine Markers in Liver Metastases of Cancers of Unknown Primary (94th Annual Meeting of the United States and Canadian Academy of Pathology, San Antonio, Texas, March 2005)

92. Rubio-Viqueira, B., Jimeno, A., Iacobuzio-Donahue, C., **Maitra, A.**, Bouraoud, N., Yeo, C., Altiok, S., Hidalgo, M. Novel *in vivo* model for drug development in pancreas cancer (96th Annual Meeting of the American Association for Cancer Research, Anaheim, CA, April 2005)

93. Hansel, D.E., Wehner, S., Herzog, V., **Maitra, A.** Alterations in Gene Expression in Pancreatic Cancer Cell Lines Induced by the N-terminal Fragment of APP (sAPP) (96th Annual Meeting of the American Association for Cancer Research, Anaheim, CA, April 2005)

94. Shi, C., Chandrashekharan, A., Thuluvath, P.J., Wistuba, I.I., Karikari, C., Argani, P., Goggins, M.G., **Maitra, A.**, Eshleman, J.R. Ultrasensitive Detection of *KRAS2* Mutations in Bile and Serum from Patients with Biliary Cancer using LigAmp Technology (96th Annual Meeting of the American Association for Cancer Research, Anaheim, CA, April 2005)

95. Mullendore, M.E., Fan, X., Eberhart, C., Matsubayashi, H., Ouellette, M., Li, Y., **Maitra, A.** Notch signaling in pancreas cancer (Late-breaking abstract at the 96th Annual Meeting of the American Association for Cancer Research, Anaheim, CA, April 2005)

96. Kumar, S., Roy, I., **Maitra, A.,** Beachy, P., Khan, S.R. Targeted inhibition of hedgehog signaling by cyclopamine prodrug for advance prostate cancer (Late-breaking abstract at the 96th Annual Meeting of the American Association for Cancer Research, Anaheim, CA, April 2005)

97. Rakheja, D., Leos, N., Mullendore, M., **Maitra. A.**, Margraf, L. Mutation of Mitotic Spindle Checkpoint Gene BUB1B in Mosaic Variegated Aneuploidy (Joint Meeting of the Society for Pediatric Pathology/Pediatric Pathology Society, Tours, France, September 2005)

98. Brune, K.A., Abe, T., Canto, M.I., O'Malley, L., Klein, A.P., **Maitra, A.**, Adsay, N.V., Fishman, E., Cameron, J.L., Yeo, C.J., Kern, S.E., Goggins, M.G., Hruban, R.H. Multifocal Neoplastic Precursor Lesions Associated with Lobular Atrophy of the Pancreas in Patients Having a Strong Family History of Pancreatic Cancer (Poster presentation at the 95th Annual Meeting of the United States and Canadian Academy of Pathology, Atlanta, Georgia, February, 2006)

99. Cao, D., Adsay, N.V., **Maitra, A.**, Antonescu, C., Klimstra, D., Hruban, R.H. Expression of c-kit in Solid Pseudopapillary Tumor of the Pancreas (Poster presentation at the 95th Annual Meeting of the United States and Canadian Academy of Pathology, Atlanta, Georgia, February, 2006)

100. Zhou, S., Kassauei, K. Poeta, M.L., Cutler, D.J., Koch, W., Sidransky, D., **Maitra, A.**, Califano, J. Detection of mitochondrial DNA mutations in head and neck cancers (Poster presentation at the 97th Annual Meeting of the American Association for Cancer Research, Washington, DC, April 2006)

101. Kristiansen, T., Gronborg, M., Harsha, H.C., Goggins, M.G., **Maitra, A.**, Pandey, A. Quantitative proteomics for identification of membrane proteins as biomarkers for pancreatic cancer (Poster presentation at the 97th Annual Meeting of the American Association for Cancer Research, Washington, DC, April 2006)

102. Weekes, C.D., Rubi-Viquera, B., Zhang, X., Jimeno, A., **Maitra, A.,** Hidalgo, M. Stromal derived factor 1 alpha mediates resistance to mTOR inhibition by the preservation of hypoxia inducible factor 1 alpha expression (Platform presentation at the 97th Annual Meeting of the American Association for Cancer Research, Washington, DC, April 2006)

103. Jimeno, A., Rubio-Viquera, B., Peralba, J., Zhang, X., Bouraoud, N., Cusatis, G., Chan, A., Singh, S., Hirsch, F., Mills, G., Kulescza, P., Altiok, S., Iacobuzio-Donahue, C., **Maitra, A.**, Hruban, R., Hidalgo, M. Combined targeted therapy shows increased efficacy in a novel in vivo pancreas cancer model (Poster presentation at the 97th Annual Meeting of the American Association for Cancer Research, Washington, DC, April 2006)

104. Jagadeeswaran, R., Ahmed, S., Janamanchi, V., Surawska, H., Karikari, C., **Maitra, A.**, Salgia, R. c-met Receptor tyrosine kinase: a novel molecular therapeutic target for the treatment of pancreatic cancer (Poster presentation at the 97th Annual Meeting of the American Association for Cancer Research, Washington, DC, April 2006)

105. Hidalgo, M., Rubio-Viqueira, B., Weekes, C., Song, D., Shah, P., Messersmith, W., Messersmith, W., Altiok, S., Kulesza, P., **Maitra, A.**, Jimeno, A. (Platform presentation at the 2006 ASCO Annual Meeting)

106. Rubio-Viqueira, B., Mezzadra, H., Iacobuzio-Donahue, C., Jimeno, A., Zhang, X., **Maitra, A.**, Altiok, S., Hidalgo, M. Optimizing targeted agents development in pancreatic cancer: A fine-needle aspirate biopsy (FNAB) based ex vivo and in vivo assay (Platform presentation at the 2006 ASCO Annual Meeting)

107. Alvarez, H., Riggins, G., Roa, J.C., Diaz, A., Pimentel, F., Ibanez, L., **Maitra, A.**, Corvalan, A.H. Serial analysis of gene expression identifies novel candidate markers for gallbladder cancer (Poster presentation at the 2007 ASCO Gastrointestinal Cancers Symposium)

108. Krizman, D., Darfler, M., **Maitra, A.**, Hood, B., Conrads, T. Proteomic identification of biomarkers of precursor lesions of pancreatic cancer (Poster presentation at the 98th Annual Meeting of the American Association for Cancer Research, Los Angeles, CA, April 2007)

109. Alvarez, H.A., Corvalan, A., Argani, P., Roa, J.C., Diaz, A., Pimentel, F., Ibañez, L., Riggins, G., **Maitra, A.** Transcriptomic profiling in a multiethnic gallbladder cancer study identifies novel candidate genes for targeted therapy (Poster presentation at the 98th Annual Meeting of the American Association for Cancer Research, Los Angeles, CA, April 2007)

110. Tsuji, K., Yang, M., Jiang, P., **Maitra, A.**, Kausha, S., Yamauchi, K., Katz, M.H., Moossa, A.R., Hoffman, R.M., Bouvet, M. Common bile duct injection as a novel method for establishing RFP-expressing human pancreatic cancer in nude mice (Poster presentation at the 98th Annual Meeting of the American Association for Cancer Research, Los Angeles, CA, April 2007)

111. Kwei, K.A., Bashyam, M., **Maitra, A.**, Van de Rijn, M., Montgomery, K., Pollack, J.R. Role of SMURF1 amplification in pancreatic oncogenesis (Poster presentation at the 98th Annual Meeting of the American Association for Cancer Research, Los Angeles, CA, April 2007)

112. Karanjawala, Z.E., Illei, P.B., Ashfaq, R., Infante, J., Murphy, K., **Maitra, A.**, Goggins, M., Hruban, R.H. New Markers of Pancreatic Cancer Identified through Differential Gene Expression (Poster presentation at the 96th Annual Meeting of the United States and Canadian Academy of Pathology, San Diego, CA, March 2007)

113. Cheung, W.L., Krizman, D.B., Alvarez, H., Hood, B.L., Darfler, M.M., Veenstra, T.D., Mollenhauer, J., Habbe, N., Feldman, G., **Maitra, A.** Application of a Global Proteomic Approach to Archival Precursor Lesions: Upregulation of DMBT-1 and TG2 in Pancreatic Cancer Precursors (Poster presentation at the 97th Annual Meeting of the United States and Canadian Academy of Pathology, Denver, CO, March 2008)

114. Hristov, A.C., Di Cello, F., Delos Reyes, M., Singh, M., Smail, S., Karikari, C.A., **Maitra, A.**, Resar, L.M.S. Expression of High Mobility Group A (HMGA1) Proteins in Pancreatic Ductal Adenocarcinoma (PDA). (Poster presentation at the 97th Annual Meeting of the United States and Canadian Academy of Pathology, Denver, CO, March 2008)

Patents and Inventions:

1. Novel diagnostic markers and therapeutic target for pancreatic cancer (International Application WO 03/030725)

2. EGFR polymorphism type predicts response to inhibitors of the EGFR (Patents pending)

3. Tumor markers for pancreatic endocrine neoplasms (Patents pending)

4. Widespread requirements for ligand stimulated Hedgehog pathway activity in growth of digestive tract tumors (Patents pending)

5. Genes overexpressed in pancreatic cancer as identified by a re-examination of the SAGE database (Patents pending)

6. Biocompatible "smart" nanogels as carriers for hydrophobic drugs (Patents pending)

EXTRAMURAL SPONSORSHIP

ACTIVE

R01 CA113669 (Maitra) 04/01/05-03/31/10 2.4 calendar
NIH/NCI $197,500
Hedgehog Inhibitors in Pancreas cancer
The Specific Aims of the RO1 are as follows: (1) Determine the effects of Hh pathway blockade in orthotopic xenografts derived from human pancreatic cancer using cyclopamine; (2) Study the role of Hh pathway in a syngeneic mouse model of pancreatic adenocarcinoma; (3) Determine predictive biomarkers of resistance and sensitivity to Hh inhibitors in pancreatic cancers.
Role: P.I.

R01 CA119397 (Prasad, SUNY at Buffalo) 09/01/05-08/31/10 .12 calendar
NIH/ NCI $212,002
Multifunctional nanoparticles in diagnosis and therapy of pancreatic cancer
The objective of this project is to develop hybrid ceramic-polymeric nanoparticles that can be utilized for targeted imaging and drug delivery in pancreas cancer.
Role: P.I. Subcontract

R01 CA112016 (Pollack, Stanford University) 04/01/06-03/31/11 .24 calendar
NIH $52,856
Gene Amplification and Deletion in Pancreatic Cancer
The specific aims of this project are 1) To identify and map at high resolution gene amplifications and deletions in pancreatic cancer; 2) To identify the "driver" oncogene/TSG(s) within localized regions of CAN; and 3) To determine the functional role of novel oncogenes/TSGs in pancreatic cancer development or progression.
Role: P.I. Subcontract

Merck (Maitra) 12/01/06-11/30/08 .12 calendar
Contract $184,272
Notch inhibitors in Human Pancreatic Cancer
The goal of this project is to determine the therapeutic efficacy of small molecule Notch inhibitors in preclinical xenograft models of pancreatic cancer.
Role: P.I. Contract

2P50 CA062924 (Kern) 07/01/07-06/30/12 1.92 calendar
NIH $7,946,234
SPORE in Gastrointestinal Cancer (Project 3C)
The goal of this project is to identify the genetics of precursor lesions of pancreatic cancer and develop biomarkers for biological classification and risk stratification in these lesions.
Role: P.I. Subproject

Sign Path Pharma, Inc. (Maitra) 09/18/07-09/17/08 .12 calendar
Contract $100,000
Preclinical Evaluation of Nanocurcumin in Pancreatic Cancer
The goal of this project is to evaluate the therapeutic effects of polymeric nanoparticle encapsulated curcumin (nanocurcumin) in xenograft models of pancreatic cancer.
Role: P.I. Contract

Novartis, Corp. (Maitra) 07/01/08-06/30/09 .12 calendar
Contract $91,861
Evaluation of single agent and combination LDE_{225} in preclinical models of pancreatic cancer
The goal of this project is to evaluate a new orally bioavailable Hedgehog inhibitor in pancreatic cancer.
Role: P.I. Contract

R21CA122265 (Eshleman) 04/01/07-03/31/09 .48 calendar
NIH/NCI $100,000
Novel tumor suppressor gene discovery in pancreatic cancer
The goal of this project is to functionally identify tumor suppressor genes using whole genome shRNA libraries.
Role: co-P.I.

N/A (Maitra) 01/01/08-12/31/08 0.6 calendar
Lustgarten Foundation $88,950
Targeting the Herpes Virus Entry Mediator (HVEM) as a novel therapeutic strategy in pancreatic cancer
The goal of this project is to test the approach that HVEM is a valid therapeutic target in pancreatic cancer, and that blockade of the HVEM – BTLA interaction will result in restitution of cytotoxic T cell activation in the tumoral milieu and result in tumor growth inhibition, using conditional models of HVEM activation *in vivo*.
Role: P.I.

R01 CA134767 (Nelkin) 07/01/08-06/30/13 2.4 calendar
NIH $250,000
Targeting CDK5 in Pancreatic Cancer: Mechanistic and Preclinical Development
The goal of this project is to develop CDK5 as a potential therapeutic target for the control of pancreatic cancer.
Role: co-P.I.

COMPLETED

LF01-009 (Maitra) 01/01/02 –12/31/03
Lustgarten Foundation for Pancreatic Cancer Research
Genetic basis of familial pancreatic cancer: a novel approach using whole genome conversion and oligonucleotide microarrays
The objective of this study was to examine somatic cell hybrids from patients with familial pancreatic cancer to detect germline mutations in the mono-chromosomal milieu, using HuSNP gene chips.
Role: P.I.

P50 CA62924 Pilot Project (Maitra) 04/01/02-06/30/03
NIH/NCI SPORE in Gastrointestinal Cancer
Development of a human mitochondrial genome sequencing microarray (MITOChip) as a universal tool for cancer detection
The objective of this study was to develop a sequencing microarray for detection of mitochondrial mutations in cancers and in clinical samples from cancer patients.
Role: P.I.

N/A (Maitra) 04/01/02-03/31/03
National Pancreas Foundation
The Familial Pancreatic Cancer Gene Chip: Designing a high-throughput sequencing microarray for risk assessment in familial pancreatic cancer
The objective of this study was to develop a sequencing microarray for germline mutation detection in familial pancreatic cancer kindred.
Role: P.I.

LF03-33 (Pollack) 01/01/03 –12/31/04
Lustgarten Foundation for Pancreatic Research
Locating novel pancreatic cancer genes with cDNA microarrays
The objective of this project was to perform comparative genomic hybridization on cDNA microarrays using genomic DNA from pancreatic cancer cell lines and xenografts in order to detect deletions and amplifications.
Role: Co-P.I.

N/A (Maitra) 01/01/03 - 01/31/05
Cancer Research and Prevention Foundation
Serum-based biomarkers in biliary tract cancers
The objective of this project was to develop serum ELISA for biomarkers identified using microarray-based gene expression analysis of biliary cancers
Role: P.I.

Johns Hopkins Clinician Scientist Award (Maitra) 07/01/04 - 04/30/05
Johns Hopkins School of Medicine
Oncogenic pathways in biliary cancers
The objective of this study is to identify and target oncogenic signaling pathways in biliary cancers, for potential mechanism based therapies.
Role: P.I.

RFA04-040 (Maitra) 07/01/04 - 05/31/05
Lustgarten Foundation for Pancreatic Research
Hedgehog Inhibitors in Pancreas Cancer
This grant was rescinded due to overlap with R01CA113669-01
Role: P.I.

R21/R33 CA107858-01 (Maitra) 04/01/04-11/30/05
NIH/NCI
A sequencing microarray for mitochondrial mutations
The objective of the study is to determine the feasibility of using mitochondrial mutations in pancreatic juice as a biomarker for pancreas cancer.
Role: P.I.

N/A (Maitra) 01/01/04–12/31/05
Maryland Cigarette Restitution Fund
Comprehensive array-based analysis of somatic mitochondrial mutations in smoking-related gastrointestinal tract cancers
The objective of this study is to determine the frequency of somatic mitochondrial mutations in smoking-associated gastro-esophageal and colorectal cancers arising in African American patients.
Role: P.I.

AACR-PanCAN Career Development Award (Maitra) 07/01/04-06/30/06
Notch Signaling in Pancreatic Cancer.
The specific aims are 1) To characterize the *in vitro* effects of individual Notch receptors (Notch 1-3) on growth of neoplastic and non-neoplastic pancreatic epithelial cell lines; 2) To characterize the *in vitro* effects of individual Notch ligands (Jagged and the Delta-like ligand DLL1) on growth of neoplastic and non-neoplastic pancreatic epithelial cell lines; and 3) To determine the *in vivo* effects of pharmacological or genetic manipulation of the Notch pathway in pancreatic cancer cells.
Role: P.I.

R21 CA109283-01 (Hidalgo) 01/01/05-12/31/06
NIH/NCI
Pharmacogenomics of Erlotinib.
The objective of this study is to develop pharmacogenomic determinants of Erlotinib activity.
Role: Co-Investigator

N/A (Mendell) 01/01/06-12/31/06
Lustgarten Foundation
The role of microRNA's in the pathogenesis of pancreatic cancer.
The objective of this project is to identify abnormally expressed microRNAs in human pancreatic cancers, and their functional consequences.
Role: Co-Investigator

Lustgarten Foundation (Maitra) 01/01/07-12/31/07
Synergistic targeting of the apoptotic machinery in pancreatic cancer
The objective of this proposal is to utilize small molecule inhibitors of X-linked IAP protein in combination with death receptor agonist antibodies as a novel experimental therapy for pancreatic cancer.
Role: P.I.

Lustgarten Foundation (Eshleman) 01/01/07-12/31/07
Pancreatic Cancer Tumor Suppressor Gene Discovery Using Rnai
The goal of this project is to discover new tumor suppressor genes for pancreatic cancer using a RNAi library-based approach.
Role: co-P.I.

N/A (Khan) 07/01/07-06/30/10
Flight Attendant Medical Research Institute
Inhibition of Hedgehog Signaling by Cyclopamine Prodrug for Prostate Cancer
The goal of this project is to develop prodrugs of cyclopamine that can be cleaved by PSMA, and to test these in prostate cancer xenograft models.
Role: co-P.I.

1R21 DK072532 (Maitra) 08/01/05-07/31/08
NIH/NIDDK
Hedgehog signaling in pancreatic neoplasia
The objective of this study is to determine the role of Hedgehog signaling in exocrine pancreatic injury/repair and neoplasia using a novel transgenic mouse model of ectopic Hedgehog overexpression.
Role: P.I.

TEACHING: University of Texas Southwestern Medical Center

Clinical Instruction

Residency Training			
Pediatric Autopsy and Placental Pathology	1999-2000	Prosector	Est. ~150 hours

Classroom Instruction

Medical School (2nd Year Pathology Course)	1997-2001	Lab. Instructor	20 hours/year

TEACHING: The Johns Hopkins University School of Medicine

Clinical Instruction:

Pathology Residency and Fellowship Training			
GI/Liver Pathology (on-scope training)	2002-	Attending	~200 hours/yr
Gastroenterology Fellowship Training			
GI/Liver Pathology (on-scope training)	2001-	Attending	2 hours / year

Classroom Instruction:

Medical School (2nd Year Pathology Course)			
Pathology Group V (Neoplasia, GI Tract)	2001-	Lab. Instructor	20 hours / year
Impact of Genomics in Medicine (Introductory lecture to Pathology lecture series)	2003-	Lecturer	1 hour / year
Cellular Injury and Adaptation	2005-	Lecturer	2 hours/year
Residency and Fellowship Teaching Rounds	2003-	Lecturer	2 hours / year
Endocrine Neoplasms of the GI Tract			
Pediatric Disorders			
Graduate Program in Pathobiology			
Classic Papers in Hepatobiliary Diseases	2003-	Lecturer	2 hours / year
Classic Papers in Apoptosis	2005-	Lecturer	1 hour/year
Fundamentals of Clinical Oncology for Public Health Professionals (School of Public Health)			
Controversies in Pancreatic Cancer	2005	Lecturer	1 hour/year

CME Instruction:

1st Annual Current Topics in GI Pathology	2001	Lecturer	1 hour
2nd Annual Current Topics in GI Pathology	2002	Lecturer	1 hour
3rd Annual Current Topics in GI Pathology	2003	Lecturer	1 hour
4th Annual Current Topics in GI Pathology	2004	Lecturer	1 hour
5h Annual Current Topics in GI Pathology	2005	Lecturer	1 hour
6h Annual Current Topics in GI Pathology	2006	Lecturer	1 hour
7h Annual Current Topics in GI Pathology	2007	Lecturer	1 hour

Mentoring

Donna Hansel, MD, PhD	2002-03	Post-doctoral Trainee;5/03-Recipient of Pathology Young Investigator Award
	2003-04	Residency Research Advisor
Surajit Dhara, PhD	2003-04	Postdoctoral trainee
Indrajit Roy, PhD	2003-05	Postdoctoral trainee
Guo-Ping Sui, MD	2003-05	Postdoctoral trainee, (International Collaborative Genetics Training Program, NIH)
Sharon Swierczynski, MD, PhD	2003	Residency Research Advisor; 5/03- Recipient of Pathology Young Investigator Award
Denfeng Cao, MD, PhD	2003-04	Residency Research Advisor
Eric Powell, MD	2004	Clinical/Research Fellowship
Robert Beaty, PhD	2004-06	Postdoctoral trainee
Arjun Chandrasekharan	2004	Pre-collegiate summer student; Research resulted in Platform presentation at USCAP Annual Mtg. (San Antonio, 2005)
Georg Feldmann, MD	2005-	Postdoctoral trainee
Hector Alvarez, MD	2005-	Predoctoral trainee, (Registered for PhD with Catholic University, Santiago, Chile)
Marcelo Reyes Marcelo Del Reyes	2005, 2006	Howard Hughes Summer Internship (University of Guam)
Gang-Ming Zou, PhD	2006-	Postdoctoral trainee
Savita Bisht, PhD	2006-	Postdoctoral trainee
Kwang Hyuck Lee, MD	2006-08-	Postdoctoral trainee
Nils Habbe, Ph.D.	2007	Postdoctoral trainee
Jan-Bart Koorstra	2007	Visiting medical student
Ji Kon Ryu	2007	Postdoctoral trainee

CLINICAL ACTIVITIES

Certification:
Fellow, American Board of Pathology (2001)

Licensure

Maryland Board of Physicians	D0057031	Expires 09/2009

Service Responsibilities

Gastrointestinal Mucosal Biopsy Pathology service	20% service commitment

ORGANIZATIONAL ACTIVITIES

Professional Memberships:

1997-	United States and Canadian Academy of Pathology
1998-2004	Society for Pediatric Pathology
2002-03	American Gastroenterological Association
2002-	American Society for Investigative Pathology
2003-	American Association for Cancer Research
2002	American Society for Clinical Oncology

Editorial Activities:
Associate Editor, *Current Molecular Medicine* (2000-2006)
Section Editor, *Laboratory Investigation* (2006 -)
Editor-in-Chief, *Current Molecular Medicine* (2006-) **(Impact Factor** 4.94)
Editorial Board, *Pancreatology* (2007-)

Ad-hoc reviewer for peer reviewed publications:
American Journal of Pathology, American Journal of Surgical Pathology, Cancer Research, Clinical Cancer Research, Oncogene, American Journal of Gastroenterology, Journal of Surgical Oncology, World Journal of Surgical Oncology, Journal of Molecular Diagnostics, Oncology (Karger), International Journal of Gastrointestinal Cancer, Journal of Clinical Pathology, Gastroenterology, British Journal of Cancer

Ad-hoc reviewer for study sections and granting agencies:
1. National Institutes of Health Study Sections:
a. Ad Hoc reviewer, National Cancer Institute Special Emphasis Panel on Centers for Cancer Nanotechnology Excellence ZCA1 GRB-S (CCNE) 2005
b. Ad Hoc reviewer, National Cancer Institute Oncology Fellowship ZRG1 F09S 2006
c. Ad Hoc reviewer, National Cancer Institute Drug Discovery and Molecular Pharmacology (DMP) study section, June, 2006
d. Ad Hoc reviewer, National Cancer Institute Basic Mechanisms of Cancer Therapy (BMCT) study section, June 2007
e. Ad Hoc Reviewer, National Cancer Institute Special Emphasis Panel ZRG1 ONCS 02
f. Ad Hoc Reviewer, National Cancer Institute Special Emphasis Panel Cancer Biology and Therapy ZRG1 ONC-U (92)
g. Ad Hoc Reviewer, National Cancer Institute Special Emphasis Panel Molecular Tumorigenesis ZRG1 ONC-K (03)
h. Ad Hoc Reviewer, National Cancer Institute Special Emphasis Panel 2008/10 ZRG1 ONC-S (03) M

2. Non-federal Granting Agencies
a. Italian Association for Cancer Research (AIRC) (2004-05)
b. Irish Research Council for Science, Engineering and Technology (IRCSET) EMBARK Initiative (2005)
c. Pancreatic Cancer Action Network (PanCAN) Pilot Grant Awards Review Committee 2008
d. Scientific Advisory Board Member, Michael Rolfe Foundation for Pancreatic Cancer Research 2007-present

Institutional commitments:
Cellular and Molecular Medicine Oral Exam Committee, October 2005 (Harshan Pisharath, CMM candidate)
Thesis Committee, David Wang (CMM candidate)

Co-Director, 6th Annual Current Topics in GI Pathology, October 2006
Co-Director, 7th Annual Current Topics in GI Pathology, November 2007

RECOGNITION

Awards

1997	Texas Society of Pathologists **John D Rainey Memorial Award**
1997	Society for Pediatric Pathology **Gordon Vawter Award**
1999	American Society of Cytopathology **Warren R Lang Award**
2000	Society for Pediatric Pathology **Lotte Strauss Award**
2001	Society for Pediatric Pathology **Harry Neustein Award**
2001	**Best Small Group Teaching Award,** UT Southwestern Sophomore Course
2004	United States and Canadian Academy of Pathology **Benjamin Castleman Award**

2006 **Maryland Outstanding Young Scientist Award** (Allan C. Davis Medal)
2007 **Eugene Di Magno Presidential Award for Junior Faculty**, American Pancreatic Association
2008 **Ramzi Cotran Young Investigator Award**, United States and Canadian Academy of Pathology

Invited Lectures

1. **Molecular mechanisms involved in PanIN progression towards invasive pancreatic adenocarcinoma** at the Annual Meeting of the Japanese Forum for Carcinoma in Situ of the Pancreas, Nagoya, Japan, October 2002.
2. **Molecular genetics of pancreatic intraepithelial neoplasia** at the Arizona Cancer Center, Tucson, AZ, November, 2002.
3. **Diseases of the Pancreas** at Georgetown University Medical School (Pathology Sophomore Course), Georgetown, DC, March 2003.
4. **Sequencing the human mitochondrion using CustomSeq resequencing microarrays** at the European Society of Human Genetics Symposium Affymetrix Users Group Meeting, Birmingham, UK April 2003.
5. **Sequencing the human mitochondrion using CustomSeq resequencing microarrays** at the American Society of Human Genetics Symposium Affymetrix Users Group Meeting, Los Angeles, October 2003.
6. **Emerging concepts: Pancreatic cancer pathogenesis** at "*Meet the Professor*" Session, American Society of Clinical Oncology Annual meeting, New Orleans, June 2004
7. **Biliary tract cancer: molecular pathogenesis and cellular targets** at the International Workshop in Biliary Tract Cancer, Shanghai, China, July 2004 (organized by NCI Division of Cancer Epidemiology and Genetics).
8. **Pancreatic Intraepithelial Neoplasia** at the Sidney Kimmel Comprehensive Cancer Center Translational Research Conference, Johns Hopkins University, September 2004
9. **Pancreatic Cancer 2005: Advances and Challenges** at the 94th Annual Meeting of the United States Canadian Academy of Pathology Advanced Molecular Pathology Course, San Antonio, March 2005
10. **Pancreatic Intraepithelial Neoplasia** at Yale University Department of Pathology Grand Rounds, March 2005
11. **Towards Effective Management of Pancreatic Cancer: New Concepts in Organ Site Research** at the 96th Annual Meeting of the American Association for Cancer Research, Anaheim, CA, April 2005
12. **Molecular Correlates of Pancreatic Intraepithelial Neoplasia** at the American Gastroenterology Association's Digestive Diseases Week, Chicago, May 2005
13. **Familial Pancreatic Cancer** at the Annual Meeting of the Lustgarten Foundation for Pancreatic Cancer Research, Memorial Sloan Kettering Cancer Center, New York, June, 2005
14. **Pathogenesis of Biliary Tract Cancer** at Yonsei University, Seoul, South Korea, August, 2005
15. **Anatomic Pathology Visiting Professor Lecture Series** at the University of Texas Southwestern Medical Center, Dallas, Texas, August 2005
16. **Monitoring Genomic stability in human embryonic stem cells** (Affymetrix Webinar), October 31, 2005
17. **Pancreatic Cancer 2006** at Department of Pathology, Northwestern University School of Medicine, Chicago, January 2006
18. **Pancreatic Cancer: Advances and Challenges** at Uniformed Services University of the Health Sciences/United States Military Cancer Institute Joint Symposium, Bethesda, March 2006
19. **Novel Molecular Approaches for Early Detection of Pancreatic Cancer** at the Annual Meeting of the Lustgarten Foundation for Pancreatic Cancer Research, University of North Carolina, Chapel Hill, June 2006
20. **Morphogenesis of Pancreatic Cancer – the role of PanIN lesions** at the International Meeting of Cancer of the Pancreas, Ulm, Germany, September 2006
21. **Pancreatic cancer: strategies for early detection and prevention** at the 2006 AACR Frontiers in Cancer Prevention and Research Conference, Washington, DC November 2006
22. **Developmental Signaling Pathways in Pancreatic cancer** at Cardinal Bernadin Cancer Center, Loyola University School of Medicine, February 2007
23. **Developmental Signaling Pathways in Pancreatic cancer** at University of California San Francisco, San Francisco, March 2007
24. **Molecular Pathogenesis of Pancreatic cancer** at Digestive Disease Week, Washington DC, May 2007

25. **A functional assay for pancreatic cancer stem cells** at American Pancreatic Association-Lustgarten Foundation for Pancreatic Cancer Research Special Symposium on Pancreatic Cancer Stem Cells, Chicago, November 2007
26. **New therapeutic targets for Pancreatic Cancer** at the Han-Mo Koo Memorial Seminar Series at Van Andel Research Institute, Grand Rapids, Michigan, April 2008
27. **Pancreatic Cancer 101** at the Pancreatic Cancer Action Annual Advocacy Day, Washington, DC March 2008
28. **Pancreatic Cancer 2008** at the Department of Pathology Grand Rounds at Georgetown University Medical Center, Washington DC, March 2008
29. **New Therapeutic Targets for Pancreatic Cancer** at Lombardi Cancer Center Visiting Professor Series, Georgetown University Medical Center, Washington DC, May 2008
30. **New Therapeutic Targets for Pancreatic Cancer at the Pancreatic Cancer Research Team Annual Meeting (Keynote Speaker)**, American Society of Clinical Oncology, Chicago IL, May 2008

APPENDIX B

Drip Column References

1. Innova Biosciences Protocol

    a. Section 2.5.3 Elution step last paragraph

    "If a drip column format is used, the displacer is allowed to pass into the column bed and the flow is then halted (e.g. by capping the column outlet). After a period of equilibration (15-30 minutes) the dissociated proteins are flushed out by application of more elution buffer. This step can be repeated until protein is absent from the eluted fractions."

2. U.S. Patent No.: 5,336,412

    a. Background of the Invention

    "Conventional gel-chromatography "drip" columns..."

3. Thermo Scientific

    a. Product catalog description

    "The easy-to-use Zeba Spin Format dramatically improves results over standard drip-column methodologies, eliminating the need to wait for samples to emerge by gravity flow and the need to monitor fractions for protein recovery."

4. CDNA Library Protocols by Ian G. Cowell & Caroline A. Austin, Humana Press, p 46, Published 1996: ISBN:089603383X

    a. Drip column preparation.

Innova Biosciences

GTP-agarose resin (γ-phosphate-linked): Low substitution (1-2 μmol/ml) High substitution (>6 μmol/ml)

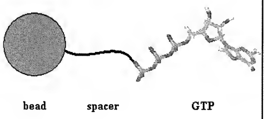
Release 002; Jan 2005 Technical bulletin 261

1. INTRODUCTION

Affinity resins have been widely used for the purification of enzymes and other proteins that bind nucleotides and related molecules.

GTP-agarose resin comprises GTP attached to agarose beads via its γ-phosphate. Two forms of the resin are available with low and high ligand substitution. A long hydrophilic spacer (14-atom) is used to minimise unwanted hydrophobic interactions and to facilitate unhindered interactions with biomolecules. The ligand is coupled through the γ-phosphate group which means that the resin is resistant to phosphatases found in many crude tissue extracts.

Fig 1 Schematic diagram of GTP agarose



2. INSTRUCTIONS

2.1. Storage of GTP agarose resin

The resin is supplied as 50% (v/v) slurry in 10mM Tris/300 mM NaCl/1mM EDTA, pH 8.0. The product is shipped at ambient temperature but should be stored at 4°C upon arrival.

2.2. Materials required (but not supplied)

For small sample volumes you may need only a microfuge and 1.5 ml tubes. For larger volumes (up to 20 ml) purification of binding proteins is conveniently carried out using disposable polypropylene columns. A simple mixing device (e.g. rotary shaker or end-over-end mixer) may also be useful.

2.3. Overview of procedure

GTP-agarose resin is added to a crude protein extract and the suspension is gently mixed. After a period of incubation the resin is transferred to a disposable column and washed to remove non-bound or loosely adsorbed material. Finally, the column is eluted with buffer containing a competing ligand.

Since GTP-agarose resin may capture more than one type of GTP-binding protein the instructions below provide only general guidance on the use of resin. You may need to modify the conditions to facilitate the binding of your particular biomolecule of interest.

2.4 Buffers

For simplicity we would recommend that you start with the same buffer for the equilibration, binding and wash steps. The elution buffer is prepared by adding a competing ligand.

2.4.1 Types of buffers

The buffer and pH must be compatible with the biomolecule of interest. Tris (pH 7.5-8.5) and Hepes (pH 7.0-8.0) are commonly used but other buffers may also be suitable.

2.4.2 Metal ions

Binding proteins often recognise metal ion-nucleotide complexes rather than free nucleotide. It may be necessary therefore to include $MgCl_2$ or other suitable metal salt (usually at least 10 mM) in column buffers to facilitate metal-dependent interactions with the resin.

2.4.3 Salts

To prevent non-specific electrostatic interactions with the matrix it is usual to include 100mM–500mM NaCl, KCl or other salt in the buffer.

2.4.4 Thiols

Thiols are often included in buffers to prevent oxidation of cysteine residues. A final concentration of 1 mM DTT is commonly used. DTT is not stable and should be added to the buffer immediately before use.

2.4.5 Protease inhibitors

Protease inhibitors (e.g. PMSF, benzamidine) may or may not be required, depending on the sensitivity of the protein of interest to proteolysis. It is also advisable to carry out the binding and wash steps in a cold room or fridge using ice-cold buffers.

2.4.6 Detergents

Detergents (e.g. Triton X-100) are sometimes used to prevent non-specific hydrophobic interactions. Since the resin and spacer are hydrophilic a detergent may not be necessary. However, if a detergent is required try relatively low concentrations (0.02-0.1%) in the first instance.

2.5 Chromatography steps

Make sure the resin has been fully equilibrated with the column equilibration buffer before commencing the purification procedure. Dialyse or desalt the sample into the same buffer before application to the resin.

2.5.1 Binding step

If you do not have access to an automated chromatography system, a batch-binding method may be used. Protein samples with volumes of 0.5-1.0 ml should be incubated in 1.5ml tubes with 50-100 µl of agarose resin. For larger sample volumes the incubation should be carried out in 10 ml, 30 ml or 50 ml tubes (or in a capped disposable column with an integral upper reservoir). Allow at least 1 hour at 4°C for binding to take place, and agitate the sample at regular intervals to prevent settling of the resin.

If you have a pump system the recommended flow rate in the first instance is 0.1-0.25 ml/min for columns that are 1-5ml in size though, you may wish to explore higher flow rates especially if the volume of material to be processed is large.

2.5.2 Wash step

If incubations have been carried out in small tubes, the resin should be subjected to five or more cycles of washing and centrifugation (e.g. in a microfuge for 3-4 seconds) using ice-cold buffers. On a larger scale it is easier to transfer the suspension to a disposable polypropylene column and to allow the non-bound material to drip through under the force of gravity. Add the wash buffer carefully down the inner surface of the column and try not to disturb the resin otherwise the wash buffer will mix with the non-bound material, leading to less efficient washing of the resin. It is important to remove all of the non-bound material prior to elution. The absence of protein in the washes is easily verified with a dye-based protein detection reagent (e.g. Bradford reagent) or with a UV monitor.

2.5.3 Elution step

It is important to appreciate in affinity chromatography that the eluting ligand (competing ligand or 'displacer') does not usually drive the bound protein from the resin; rather, it associates with proteins that dissociate from the resin and prevents their rebinding. The concentration of the displacer has to be sufficiently high to compete with any unoccupied ligand sites on the resin and sufficient time has to be allowed for dissociation to take place. Resins with a high ligand density (8-12 µmol/ml; 8-12 mM) may need a higher concentration of competing ligand for efficient elution than resins with a low density (1-2 µmol/ml; ~1-2 mM). If GTP is employed as the competing ligand a concentration in the range 5-10 mM is a useful starting point.

For experiments carried out in 1.5 ml tubes, the elution buffer (0.25-1.0 ml) is added to the resin.

After >30 minutes the resin is centrifuged and the supernatant fraction is carefully removed. If a drip column format is used, the displacer is allowed to pass into the column bed and the flow is then halted (e.g. by capping the column outlet). After a period of equilibration (15-30 minutes) the dissociated proteins are flushed out by application of more elution buffer. This step can be repeated until protein is absent from the eluted fractions. If a pump is available the column can be eluted using continuous flow at a rate of 0.05-0.1 ml/min, but it may be necessary to reduce the flow rate (or switch off the pump for a period of time) to ensure that the protein elutes in a relatively small volume.

2.5 Column regeneration

After each run, wash the column with a neutral buffer containing 1M NaCl and then re-equilibrate with 10 mM Tris/300 mM NaCl, 1 mM EDTA pH 8.0. Do not wash the column with strong acid or base. For long-term storage add a preservative (e.g. 0.1% sodium azide).

3. Ordering information

504-0002	2 ml* low ligand density
504-0005	5 ml* low ligand density
505-0001	1 ml* high ligand density
505-0002	2 ml* high ligand density

* packed volume

For bulk quantities or other densities of ligand please contact our customer service department.

DOCUMENT CENTER HIDE

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Password:

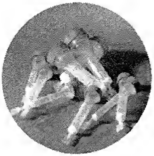
LOG IN

- I Forgot My Password
- New User Registration
- Why Register?

SHOPPING CART HIDE

- **Ordering Information**
  - Shipping Info
  - Credit Info
  - Returns Info
- **Current Order**
  - Currently Empty
  - Order Form

Zeba Spin Desalting Columns



Why desalt protein samples using cumbersome methods that deliver mediocre results?

Desalt sample volumes ranging from 2 µl to 4 ml with Zeba Desalting Columns and experience exceptional protein recovery quickly

Although numerous techniques end resins for desalting ere aveilable, most have many drawbecks, including significant sample loss, long processing times and the need to collect multiple fractions. Zeba Desalting Columns* provide excellent protein recovery without the limitations associated with other desalting methods. With the introduction of Zeba Desalting Columns in 2, 5 and 10 ml formats to complement the Micro and 0.5 ml versions, the Zeba Deselting family of products allows processing of samples volumes ranging from 2 µl to 4 ml (Table 1).

The easy-to-use Zeba Spin Format dramatically improves results over standard drip-column methodologies, eliminating the need to wait for samples to emerge by gravity flow and the need to monitor fractions for protein recovery. Zeba Desalting Columns require no chromatographic system, cumbersome column preparation or equilibration and they can process multiple samples in ~8 minutes.

Zebe Desalting Columns contain a proprietary high-performance desalting resin, exclusive to Pierce, thet offers exceptional desalting and protein-recovery characteristics compared to other commercially available resins (Figure 1). Samples containing as low as 25 µg/ml of protein can be processed, providing exceptional protein recovery and ≥ 95% retention of salts and other small molecules (< 1,000 MW). Zeba Spin Desalting Columns are recommended for processing compounds > 7,000 MW.

Highlights:

- Exceptional protein recovery
- No screening fractions for protein or waiting for protein to emerge by gravity flow
- Wide product offering accommodates your sample needs
- Easy-to-use with no cumbersome column preparation or equilibration
- Minimal sample dilution
- Available in formats such as spin columns end chromatography cartridges

Table 1. Recommended sample volumes for Zeba Spin Columns.

Resin Bed	Sample Volume	Zeba Part #
75 µl (micro) column	2-12 µl	89877
0.5 ml column	30-130 µl	89882
2 ml column	200-700 µl	89889
5 ml column	500-2,000 µl	89891
10 ml column	1,500-4,000 µl	89893

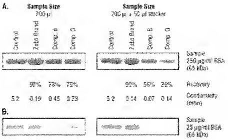


Figure 1. Increased protein recovery with Zeba Spin Desalting Columns. Samples of bovine serum albumin (BSA) at Figure 1A. 250 µg/ml and Figure 1B. 25 µg/ml in 1 M NaCl were desalted with the 2 ml Zeba Desalting Columns and other commercial desalting resins using similar formats. A portion of the recovered sample (10 µl) was analyzed by SDS-PAGE. The remaining sample was used for conductivity measurements and BCA Protein Assay* (Product # 23225) to determine protein concentration. Zeba Desalting Resin provides significantly greater protein recovery under all conditions tested. Conductivity and protein recovery values after desalting are indicated for 250 µg/ml samples.

Related Products

Pierce Centrifuge Columns (empty)
Zeba Micro Desalting Columns
Zeba Spin Desalting Columns, 0.5 ml

* BCA Technology is protected by U.S. patent # 4,839,295. U.S. patent pending on Zeba Micro Column Technology.

Ordering Information

Certificate of Analysis Instruction Book with Protocols MSDS

Buy	Product #	Description	Pkg. Size	Files	Price
Add	89882	Zeba Spin Desalting Columns, 0.5 ml	25/pack		$97.00
Add	89889	Zeba Spin Desalting Columns, 2 ml for 200 - 700 µl samples	5 columns		$38.00
Add	89890	Zeba Spin Desalting Columns, 2 ml for 200 - 700 µl samples	25 columns		$178.00
Add	89891	Zeba Spin Desalting Columns, 5 ml for 600 - 2,000 µl samples	5 columns		$49.00
Add	89892	Zeba Spin Desalting Columns, 5 ml for 600 - 2,000 µl samples	25 columns		$222.00
Add	89893	Zeba Spin Desalting Columns, 10 ml for 1,500 - 4,000 µl samples	5 columns		$59.00
Add	89894	Zeba Spin Desalting Columns, 10 ml for 1,500 - 4,000 µl samples	25 columns		$265.00
Add	89934	Pierce Chromatography Desalting Cartridges See page for all Pierce Chromatography Cartridges	5 x 1 ml		$138.00
Add	89935	Pierce Chromatography Desalting Cartridges See page for all Pierce Chromatography Cartridges	5 x 5 ml		$158.00
Add	89883	Zeba Spin Desalting Columns, 0.5 ml	50/pack		$178.00

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US005336412A

United States Patent [19]

Huse et al.

[11] Patent Number: 5,336,412

[45] Date of Patent: Aug. 9, 1994

[54] PUSH COLUMN CHROMATOGRAPHY METHOD

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[73] Assignee: Stratagene, La Jolla, Calif.

[21] Appl. No.: 84,534

[22] Filed: Jun. 28, 1993

Related U.S. Application Data

[60] Division of Ser. No. 827,995, Jan. 30, 1992, which is a continuation of Ser. No. 292,808, Jan. 3, 1989, abandoned.

[51] Int. Cl.5 .... B01D 15/08

[52] U.S. Cl. 210/635; 210/656; 536/25.4

[58] Field of Search 536/25.4; 210/635, 656, 210/198.2, 416.1, 472; 604/187, 190, 191; 436/161, 178; 422/70, 100, 101; 73/864.16, 864.17, 864.18, 864.81, 864.82, 864.83, 864.85, 864.86, 864.87

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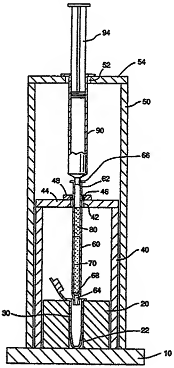
Attorney, Agent, or Firm—Limbach & Limbach

[57] ABSTRACT

A method for chromatography of DNA, RNA, proteins and other molecules includes the use of a column adapted to hold a chromatography material and a sample to be filtered. A pneumatic pressure differential is applied across the column and the sample is urged through the chromatography material. A selected portion of the sample may then be collected.

3 Claims, 2 Drawing Sheets



PUSH COLUMN CHROMATOGRAPHY METHOD

This application is a divisional of allowed application Ser. No. 07/827,995, filed Jan. 30, 1992, which is a continuation of application Ser. No. 07/292,808, filed Jan. 3, 1989, now abandoned.

BACKGROUND OF THE INVENTION

1. Field Of The Invention

The present invention relates to an apparatus and methodology for the chromatography of materials, and in particular, chromatography based on molecular size, affinity and the like as used, for example, in the purification, separation or isolation of DNA and RNA fragments, proteins and other molecules.

2. Background Art

Removing unincorporated nucleotides from DNA and RNA fragments, isolating RNA fractions, purifying proteins and other macromolecules, are important procedures having a variety of applications. In DNA and RNA synthesis, unincorporated nucleotides must often be removed when constructing nicktranslated probes, RNA probes and end-labeled oligonucleotides, as well as "filled-in" DNA fragments. It is important to separate the unincorporated free-nucleotides from the labeled probe as unincorporated label may bind to the solid support, resulting in unacceptably high levels of background noise. Isolation of RNA fractions may be employed in the separation of, for example, polyadenylated RNA from nonpolyadenylated RNAs. The use of chromatography methods to isolate and identify proteins and other macromolecules is another well known application.

Current chromatography methods, used particularly in connection with DNA and RNA synthesis, include ion-exchange chromatography, several variations of gel chromatography and others. Each has its own disadvantages. For example, ion-exchange methods require a number of steps which may result in a significant investment of time and, in the case of radiolabeled nucleotide filtering, extensive handling of radioactive material. Conventional gel-chromatography "drip" columns are tedious, requiring time to both pour and run. Spin columns, a variation of the "drip" column, are somewhat faster, but risk radiation exposure and contamination in the case of radionucleotide chromatography, and may yield less reliable results.

An alternative chromatography approach which avoids the aforementioned difficulties would therefore be desirable.

SUMMARY OF THE INVENTION

The present invention is directed to an apparatus and method for purifying, isolating and separating materials using gel chromatography. To that end, a chromatography material and a sample may be loaded into a column and pneumatic pressure applied to urge the sample through the chromatography material, whereby portions of the sample may be collected by the chromatography material and other portions excluded. In one embodiment, a positive pneumatic pressure is provided and in a second embodiment a negative pressure is applied. Additionally, a novel support structure may be employed to support the column during chromatography. The sample may thus be quickly and reliably treated.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 is an exploded perspective view of an apparatus constructed in accordance with the present invention comprising a column, pressure inducing means, a collection vial and associated support structure.

FIG. 2 is a cross-sectional view of the apparatus of FIG. 1 in a loaded position.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Referring to FIGS. 1 and 2, a chromatography apparatus constructed in accordance with the present invention comprises a generally disk shaped base 10 having a pair of retainers 12 and a generally cylindrical vial holding assembly 20 mounted thereon. Centrally located in the vial holding assembly 20 is a cylindrical chamber 22 for supporting a collection vial 30, into which the eluent from the column may be collected. The vial 30 may be a decapped Eppendorf tube or other suitable collection means. Removably mounted to the base 10, and slideably engaging the exterior wall of the vial holding assembly 20, is a generally cylindrical column support assembly 40. The column support assembly 40 includes a central aperture 42 formed in the . generally planar upper surface 44 thereof. As shown in FIG. 2, the support assembly may have a resilient collar 46, such as an "O" ring or the like, positioned circumferentially adjacent the aperture 42, and a collar retainer 48 adapted to retain the collar 46 adjacent the aperture 42. Alternatively, as shown in FIG. 1, the collar 46 and the retainer 48 may be eliminated.

Optionally, a generally cylindrical pressure inducing means support assembly 50 may be removably mounted on the base 10. The support assembly may comprise a central aperture 52 formed in the generally planar upper surface 54 thereof, and is configured to slideably engage the exterior wall of the column support assembly 40. The aperture 52 is preferably axially aligned with the aperture 42 in the column support structure 40, which itself is Preferably axially aligned with the chamber 22 in the vial holding assembly 20.

Alternatively, as shown in FIG. 1, the support assembly 50 may include an upper surface 54 having no aperture therein. The support assembly 50 may be further provided with a pair of locking tabs 54 adapted to engage the retainers 12 on the base 10 to lock the support assembly 50 in place during use. Other suitable locking mechanisms, such as threads, could also be employed. The assemblies 20, 40 and 50 may be formed of a radiation shielding material or, preferably, are constructed to fit securely inside a beta shield device. Molded plastic materials have been found suitable although other materials may also be employed.

Supported by the column holding assembly 40 above the vial 30 is a substantially tubular chromatography column 60. The column 60 may be about 1 ml in size, having a preferred internal diameter of about 5 mm and a preferred length of about 100 mm, and comprises openings 62 and 64, respectively, at each end thereof. An annular lip 66 may be provided circumferentially adjacent the upper opening 62, as shown in FIG. 1. The upper opening 62 is adapted to receive a chromatography material 70 and a sample 80 to be filtered. The lower opening 64 has an area of reduced cross-section adapted to prevent passage of the chromatography material 70 while permitting passage of the sample 80. Additionally, a screen or filter 68, comprising, for ex-

.7. Size Fractionation

There are many types of filtration media used to separate DNA molecules. ephacryl S-500 medium separates efficiently in the 2-kb size range. Drip colnns made with Sephacryl S-500 medium separate by size, the larger cDNA olecules eluting from the column first and the small unligated adapters and ncorporated nucleotides eluting later. The cDNA will not have a high numer of counts, but will be detectable by a handheld monitor at ≤250 cps.

7.1. Drip-Column Preparation

1. Discard the plunger from a 1-mL plastic syringe, and insert a small cotton plug. Push the cotton to the bottom of the syringe.
2. Fill the syringe to the top with Sephacryl S-500 filtration medium.
3. Place the syringe in a rack and allow the column to drip "dry."
4. Fill the syringe up to ~0.5 cm from the top with medium, and drip through as in step 3.
5. Rinse the column with four aliquots of 300 µL of 1X STE buffer (total wash volume of 1200 µL). Drip dry after each addition of buffer.

7.2. Collecting Fractions

1. Pipet the cDNA into the washed Sephacryl S-500 drip column, and allow to drip through. This is fraction 1. The recovery volume is ~150 µL and does NOT contain cDNA (*see* Note 6).
2. Load two more aliquots of 150 µL of 1X STE buffer on the column and drip through. These are fractions 2 and 3.
3. Collect fraction 4 in a fresh tube. Load 150 µL of 1X STE buffer and drip as before.
4. Collect fraction 5 as in step 3. Two fractions are usually adequate. The size of the cDNA decreases. Two fractions are usually adequate. The size of the cDNA decreases in each additional fraction. Most of the radioactivity will remain in the column owing to unincorporated nucleotides. Discard the radioactive drip column appropriately.
5. Remove 5 µL from each fraction (or up to $^{1}/_{10}$ of the fraction volume) for analysis of cDNA size on a 5% nondenaturing acrylamide gel. These aliquots can be frozen at –20°C.
6. To remove any residual enzyme from previous reactions, phenol-chloroform/chloroform extract (*see* Note 5).
7. Add twice the volume of 100% (v/v) ethanol to precipitate the cDNA.
8. Place on ice for 1 h or at –20°C overnight.

8. Quantitating the cDNA

1. Microcentrifuge the fractionated cDNA at maximum speed for 30–60 min at 4°C. Carefully transfer the ethanol to another tube, and monitor with a Geiger counter. Most of the counts should be present in the pellet. Discard the ethanol appropriately.